MECHANISM OF MOVEMENT OF EPIDERMIS, ESPECIALLY ITS MELANOPHORES, IN WOUND HEALING, AND BEHAVIOR OF SKIN GRAFTS IN FROG TADPOLES

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Probably no other animal tissue is more active than an epidermis in repairing lost or damaged parts. The process is not primarily one of growth. A deficiency, either large or small, in the epidermis is rapidly covered by centripetal movement of the surrounding epithelium, and later, by reorganization and growth, the original condition is restored. Rand (1915) in considering the wound reactions of actinians says (p. 207): "In general, an epithelium will not tolerate a free edge. When such an edge arises, accidentally or otherwise, the epithelium extends until, if possible, the free edge meets and unites with some other portion of the same layer or with another epithelium. The essential function of an epithelium is to cover a surface continuously."

The investigations of Fraisse (1885), Barfurth (1891), Born (1896), Morgan (1901), Loeb and Strong (1904), Rand (1905), Eycleshymer (1907), Matsumoto (1918), Loeb (1920), Arey (1925), and Collins and Adolph (1926) point unanimously to the conclusion that wounds are at first covered by movement of the surrounding epithelial cells and that proliferation occurs later to restore the original thickness of the layer.

The cause and mechanism of the cell movement are not so well agreed upon. Born (1896) believed that the cells flatten to cover a larger surface than previously. Rand (1905), however, referring to wound healing in earthworms, says (p. 46): "There certainly is little evidence in favor of supposing that the concentric advance of the epidermis is due to the tendency of the individual cell to spread itself over the greatest possible surface."

Barfurth (1891) proposed that, while the movement of the epidermis might be in part a passive "Verschiebung" due to relief from lateral pressure in the layer, it was in the main an active movement of cells which had become "embryonal beweglich (amöboid)" (p. 417). Oppel (1912) described epithelial movement as an active movement—often a "Massenbewegung"—resulting from change of form of the

epithelial cells which, however, are not ameboid. On the contrary, Holmes (1914), observing amphibian epidermis in tissue culture, decided that epithelial movement is not a "Massenbewegung" but is the result of essentially ameboid movements of individual cells. And, again, Collins and Adolph (1926) concluded that wound closure is accomplished by a mass movement or "pushing in" process of the epithelial layer and "not by the independent migration of cells" (p. 491). Loeb and Strong (1904, p. 282) considered it probable that "tension, either previously existing or called into play by the wound, is the cause" of the closure of the epidermal wounds.

Morgan (1901, p. 70), describing wounds in very young tadpoles, stated that "the wound is covered not by individual cells wandering over the exposed surface, but by a steady advance of the smooth edge of the ectoderm toward a central point. . . . As there are no muscle fibres present . . . , the result cannot be due to muscular contraction. . . ."

The artificial cultivation of epithelial tissue has given striking evidence that the cells are motile and their movement ameboid. This was suggested at an early date by Peters (1889), who studied wound closure in the cornea of frog eyes. Tissue culture studies have been carried out by Loeb (1902 and 1912), Harrison (1910 and 1914), Holmes (1913 and 1914), Oppel (1913), Uhlenhuth (1914 and 1915), Hooker (1914), Matsumoto (1918), Maximow (1925), and others. For the most part these investigators agree that movement of epithelial cells over a wound surface is ameboid and usually stereotropic.

The cause initiating the cell movement is not definitely known. Rand (1905) suggested a wound stimulus: "The most important factor in the earlier part of the process of reparation is cytotaxis; the individual columnar cells of the existing epidermis are affected by some directive stimulus and respond by an active migration, which results in the covering of the surface of injury by a protective epithelium—the first definitive step toward regeneration" (p. 52). Taube (1923) concluded that materials of injured cells flow over wound surfaces and serve as a means of chemical stimulation for the uninjured cells at the edge of the wound, causing them to migrate and later to divide. Maximow (1925) suggested that regenerative proliferation may be the result of direct stimulation by the injury or of the action of specific chemical substances. The trend of recent conclusions is in the direction of the activation of epithelial cells by a wound hormone or wound stimulus of some sort.

In the following pages are reported studies upon the skin of tadpoles of *Rana clamitans*. The studies were carried out with a view to

further understanding the movement of epidermal cells over a denuded area, the behavior of host tissue in relation to a skin graft, and especially the mechanics of radial arrangement of epidermal pigment cells around a wound.

The animals used in these experiments were collected from ponds or streams near Cambridge, Massachusetts. They were usually 70 to 80 mm. in length.

The Mechanics of Wound Closure

It has been frequently noted that epidermal wounds close very quickly. In tadpoles, skin wounds 5 to 10 mm. across may be entirely

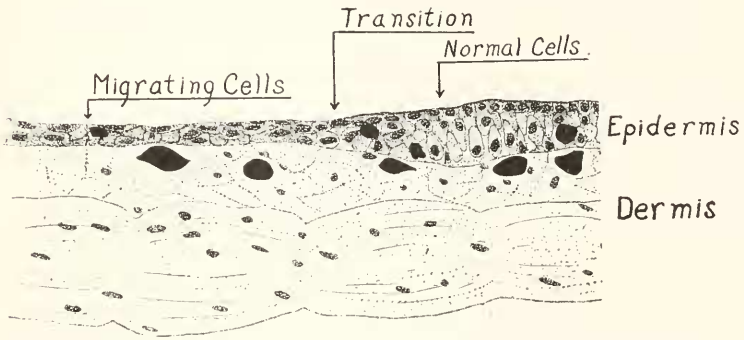

Fig. 1. Section perpendicular to the surface of skin near a wound, showing migrating cells and the transition to those not migrating. The wound was a short distance to the left of the cells illustrated.

closed in from 6 to 24 hours. Upon the occurrence of a wound, a quantity of blood always flows into the area and there coagulates. The coagulum usually nearly fills the wound temporarily, being about equal in thickness to the skin. Continuous microscopical examination, immediately following the operation, revealed that the edge of the epidermis starts to move toward the center of the wound within a few minutes after the wound is made. The epidermal layer advances over the coagulum, moving equally from all sides toward the center of the wound. If the edge of the wound is irregular, the advancing layer is correspondingly irregular in outline. Over large wounds the advancing epidermis thins out until it may be only one or two cells in thickness. The thinning may extend several millimeters distant from the wound, depending on the size of the wound. Figure 1 shows the limit of the thinned region of the layer and the transition to the normal condition which in this case is about 5 mm. from the edge of the rather large wound. The advancing cells are quite undifferentiated. Those of the

cuticular layer are hardly distinguishable from those of other layers of the epidermis.

Sections parallel to the surface of the layer were cut from normal epidermis (Fig. 2) and from areas adjacent to a closing wound (Fig. 3). In such sections the normal cells exhibited fairly regular polygonal

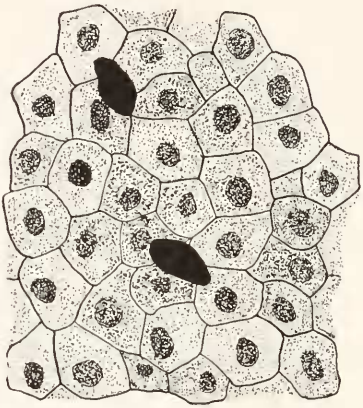

Fig. 2. Section parallel to the surface of normal epidermis. The solid black areas represent unexpanded, epidermal melanophores.

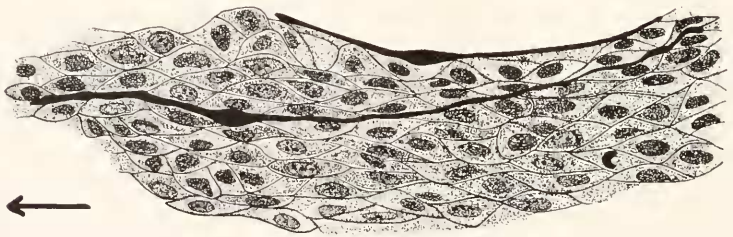

Fig. 3. Section parallel to the surface of epidermis near a wound, showing cells elongated in the direction of movement. The cells are moving in the direction of the arrow. The elongate black areas represent expanded, epidermal melanophores.

outlines while in an area near a closing wound the cells were from two to four times as long as wide, with the elongation in the direction of movement. This elongation of cells occurs at all points between the edge of the advancing layer and the region of transition to the undisturbed epidermis. Several writers have suggested that epithelial cells elongate perpendicularly to the edge of a wound, but an extensive reading of the literature discovers no mention of appropriate sections having been made to establish the fact. Sections perpendicular to the surface merely suggest the possibility of elongation.

When advancing edges of epidermis meet at or near the center of

the wound, there often takes place a piling up of cells. This piling up suggests that the causal factor for migration continues to operate for a time after the advancing edges have come together. After the wound has been covered by a layer of epithelium, the coagulum disappears. Within two or three days mitosis begins and the layer over the wound continues to increase until it is somewhat thicker than the epidermis in other parts of the body. Also the deficient epidermis surrounding the wound is built up, by cell proliferation, to the normal thickness of the layer.

In wounds made entirely through the skin the dermis is slow in closing, but the epidermis closes as usual, subsequently thickening until it is much thicker than the normal epidermis. Sometimes the thickness of the layer over a healed wound may be equal to the combined thickness of the normal epidermis and the dermis. When transplantations are made, the small gaps that necessarily exist between host skin and graft are closed by epidermis which grows in to fill the deficiency as if to maintain a smooth surface to the body. As the dermis is built up, the epidermis returns to its usual thickness. The function of epithelium may be not only "to cover a surface continuously," as declared by Rand, but also to restore a smooth external surface after small injuries.

Skin Grafts and the Compatibility of Tissues

The diversely pigmented integument of amphibians, possessing extraordinary capacity for repair and regeneration and readily amenable to grafting operations, offers many obvious advantages for the study of problems in tissue compatibility.

A patch of ventral, unpigmented skin from a tadpole was transplanted to the back of the same animal. Twenty-six autotransplants of this kind were made. In each instance the transplanted piece was 5 to 7 mm. square. Several operations were performed in which a dorsal patch of skin was merely cut loose and then reimplanted where it had previously been. Other patches were rotated through 90° or 180° and reimplanted.

The epidermis of an autotransplant united with that of the host immediately after the graft was made, but after that union had been effected there was no further movement on the part of the host tissue. In contrast to what happened in homoiotransplants, there was no invasion of the graft by host tissue. There was no sign of incompatibility between the ventral and dorsal skin tissue. Once the epidermal layers had united, the regenerative activity ceased. Dorsal pigmented patches which were rotated or reimplanted in their original positions were not disorganized and in a few weeks the limits of the grafts could not be

recognized. The half-white, half-pigmented, lateral patches which were rotated through 180° remained unchanged, the light area of the patch remaining unpigmented and the pigmented end retaining its pigment with none spreading into the surrounding white area (Fig. 4). Animals bearing this type of graft were observed for as long as 80 days. Collins and Adolph (1926), however, observed disorganization of rotated skin grafts in experiments on *Triturus*. It seems clear that no incompatibility exists between dorsal and ventral skin of an individual tadpole, although, in respect of pigment, they are locally specific.

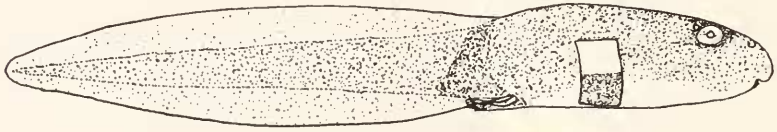

Fig. 4. Frog tadpole showing lateral, rotated graft.

Homoiotransplants were made by grafting ventral unpigmented skin from one animal onto the back of another. The grafts healed in place very rapidly so that in a few hours a microscopic inspection of the tissue in the region of the union would scarcely reveal just which were host and which were graft cells. Not all of the movement, however, in covering such gaps is accomplished by the host epidermis, for that of the patch moves almost as rapidly until the two unite. The dermal layers of host and patch are much slower in uniting than the corresponding epidermis, requiring many days or even weeks.

Sixty homoiotransplants were observed. Without exception they became occupied by host epidermal cells; and, if the animals lived long enough, dermal cells entered the unpigmented area. The grafts began to become pigmented in from 1 to 24 days after transplantation, the average being 9. The host epidermis, carrying the epidermal pigment cells, moved centripetally into the graft quite equally from all sides. Once having started, an average of 6 days elapsed before the pigment-bearing host epidermis reached the center of the patch. From a cursory examination of the surface, it was believed that the host epidermis grew over the patch, covering the epidermis as a whole. A study of microscopic sections, however, gave no indication of overgrowth or undergrowth of the epidermis, in accord with the account given by Cole (1922). The epidermis of the patch evidently undergoes destruction and is simultaneously replaced by that of the host. What appeared to be the remains of disintegrated graft cells were found in the cells at the edge of the advancing host epidermis, suggesting phagocytosis. That epidermal cells may be phagocytic at times has been stated by Loeb (1902, 1912).

The invasion of a graft by host epidermis is essentially a process of delayed wound healing. The delay is necessitated by the presence of the graft epidermis. Except for complications occasioned by the removal of the patch epidermis, the behavior of the host skin in relation to the homoiotransplant is nearly like that in ordinary wound closure. The host cells near the patch become elongated in the line of movement just as in wound healing (Fig. 3).

Fourteen transplantations were made in which patches of dorsal skin were transferred to wounds on the ventral surface of the animal. Whether the graft was an auto- or a homoiotransplant, healing took place as usual, but in the course of a few days the patch showed signs of degeneration and subsequently sloughed off until all or the greater

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FIG. 5. Photograph of corner of skin graft showing the network of enlarged blood vessels.

part was lost. It is believed that this condition was not necessarily the result of incompatibility between the tissues but that the ventral side of the body, with its very thin body wall, is an unfavorable place for a graft to receive nourishment.

BEHAVIOR OF BLOOD VESSELS IN SKIN GRAFTS

Nearly all skin transplants early become red because of the enlargement of their blood vessels. The degree of redness varies from slight traces to a deep, solid red covering the entire patch. The reddening begins as blood vessels here and there over the patch become enlarged for very short distances. The enlarged regions then extend until a close network of such vessels may be seen over any portion or all of the patch (Fig. 5). The redness becomes apparent usually the

second day after the graft is made, but it has been observed as early as 18 hours after an operation, with definite circulation in the enlarged vessels at 24 hours. The redness may persist for one to two weeks. In the greater number of cases the blood vessels enlarged until, in a few or many places in each graft, they broke to release blood into the loose dermal tissues, causing the appearance of a continuous layer of blood. This stage persisted usually less than a week. As it cleared, blood vessels of normal size could be seen, suggesting repair of the vessels. Since the vessels that occasioned the temporary redness became filled with blood within the short time of a few hours after the operation and because their arrangement is so similar to that of the vessels normally present in the ventral skin, it is probable that they are not newly developed after transplantation, but are the original vessels of the grafted skin.

Cole (1922) has described the reddening of homoiotransplants of tadpole skin but states that autotransplants do not show this reaction. He says (page 391) : " The occurrence of such a process is evidence of a rather violent chemical reaction going on between the protoplasms of the graft and host, and a merely specific difference between the two could set up the reaction." Cole's conclusions, however, are not in accord with the experiments described in the present paper, for in these experiments both auto- and homoiotransplants became reddened in nearly all cases. Since white autotransplants never became pigmented and since there was no other indication of any reaction between tissues, it seems evident that increased vascularity is not an indication of incompatibility between tissues. Furthermore, there is considerable evidence that enlargement of the blood vessels is due to mechanical stretching of their walls. As circulation becomes established in the graft, the blood exerts pressure on the walls of the vessels. In normal tissues, tonus of the vessels balances this internal pressure. There is slight variation in the caliber of normal vessels to meet the varying needs of the animal, the tonus being controlled by the nervous system. In transplanted patches the nervous connections have been cut during the operation, leaving the blood vessels of the patch without normal tonus, only a certain elasticity intrinsic to the vascular tissue itself persisting. It is highly probable, therefore, that enlargement of blood vessels following transplantation is due to stretching of the vessels from internal pressure in the absence of nervous control and not to a specific reaction between tissues as concluded by Cole.

May (1924), after transplanting lizard (*Anolis*) skin, concluded (p. 553) : " Transplants of pigmented skin lose their colour-changing

power immediately on being completely disconnected from neighbouring tissues, and regain it slowly as nerves regenerate." It has not been ascertained just when nerves regenerate to innervate the grafted tadpole skin, but the apparent resumption of control of the blood vessels agrees in time with that given by May for the regeneration of nerves in the skin of *Anolis*. May's conclusions apply very well to the apparent loss and regaining of nerve control of the blood vessels of the tadpole grafts.

Radial Arrangement of Epidermal Pigment Cells Around a Wound or Skin Transplantation

When expanded epidermal pigment cells lie upon or near a skin wound which is healing or a transplanted patch of skin which is be-

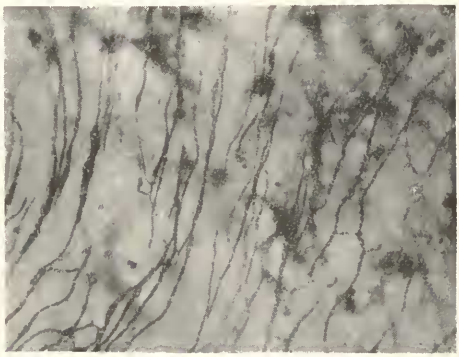

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Fig. 6. Photograph showing the nearly parallel arrangement of epidermal pigment cells near a wound.

coming pigmented, they show a particular arrangement in relation to the wound or graft. In general, the axes of elongated melanophores are perpendicular to the edge of the wound or, in other words, they are parallel to the line of movement of the epidermal cells toward the wound or graft. In any one localized area the greater number of epidermal melanophores are parallel (Fig. 6). Some cells may be at an angle to others, or occasionally a melanophore may be perpendicular to the axes of the greater number, but such cells are too few to impair the conspicuous radial arrangement of the majority. This arrangement may extend for several millimeters external to the edge of the wound or graft. The area over which radial arrangement may take place was found to coincide with the extent of the region within which migration

of the epidermal cells occurs. In Fig. 1 the limit of radial arrangement occurs at the point labeled "transition." This observation is very significant in relation to the mechanics of the radial arrangement.

It is obvious that radial position of a pigment cell will not be evident unless the cell is expanded. Radial arrangement depends upon expansion of the melanophores, but the causes of radial arrangement and expansion were found to be distinct.

Since investigation of the causes of radial arrangement could be carried on only when the epidermal melanophores were expanded, it was desirable to find an artificial means of producing expansion. The following technique was used. An injection fluid was prepared by adding desiccated bovine pituitary gland to Ringer's solution and filtering the mixture through filter paper. The powdered gland material dissolved and filtered better if the liquid were slightly warm. About one gram of desiccated gland was used in 10 cc. of Ringer's solution, but wide variations of this concentration proved to be effective. Intraperitoneal injections were made, using a small hypodermic syringe with a dosage of about 0.2 cc. After an injection of this kind the melanophores began to expand in about twenty minutes and continued expanding for one or two hours until maximal or nearly maximal expansion was reached. Allen (1917), Swingle (1921), and Collins and Adolph (1926) have studied in considerable detail the effect of pituitary gland and its extracts on pigment cells, but in the experimental work now being described the pituitary extract was used merely to expand melanophores in connection with the study of the problem of their radial arrangement.

Expanded epidermal melanophores near a wound which was being covered, or around grafts toward which epidermis was moving, exhibited more or less marked radial arrangement. Therefore radial arrangement of melanophores may be expected wherever there is a migrating epidermis whose melanophores are expanded. After a wound or graft has been covered by the surrounding epidermis and the layer has nearly returned to its normal thickness, the melanophores begin to lose their special arrangement. In homoiotransplants radial arrangement persisted from one to two weeks or even longer. In one large homoiotransplant, after it had become pigmented and the radial arrangement lost, two holes, about 3 mm. apart and each about 1 mm. across, were cut through the skin of the graft. The pigment cells were partially expanded. Twelve to eighteen hours afterward there was marked radial arrangement around each of the holes.

In considering the mechanism of radial arrangement of melanophores, the conditions of both normal and migrating cells must be kept

in mind (Figs. 2 and 3). During the migration of the epidermal cells, their space relationships are continually changing. Further, *the epidermal cells closest to the edge of the wound or graft move faster than those successively farther from the wound or graft.* In the initial advance of cells in covering the wound, the cells closest to the edge of the wound travel a greater distance than cells successively farther from the edge. It is believed that the mechanism of radial arrangement may be completely described from the facts just stated.

Unless a pigment cell is lying with its long axis exactly perpendicular to the line of movement of the epidermal cells, the end of the melanophore nearer the edge of the wound will be carried faster than the remainder of the melanophore. The carrying of one end of the cell faster than the other in a given direction will tend to rotate the comparatively long pigment cell to lie in a new position, more nearly in the line of movement of the epidermal cells. Since a single epidermal melanophore may be as long as the diameters of twenty epidermal cells, this action could easily occur. Continuous microscopic observations made on living epidermis at the edge of a wound support in every detail the suggestion just made. Such observations were made continuously on a single wound for as long as twelve hours.

Suggestions for apparatus used in making the observations just mentioned were obtained from Clark (1912). A small glass box was constructed, part of one side of which was made of "cover slip" glass to permit the use of high-power microscope objectives. The box was filled with paraffin to a depth of about 5 mm. The tube of an ordinary compound microscope was turned to the horizontal position and the glass box was secured to the mechanical stage. With the box in this position, the open side was uppermost and the side made of the thin glass was next to the objective lens of the microscope.

When a wound was to be studied, the tadpole was first anesthetized by placing it in water to which had been added a few drops of ether. As soon as anesthesia was produced, the animal was placed in the glass box, which contained a 0.05 per cent chloretone solution. Chloretone is a very slow-acting anesthetic and causes melanophores to expand. Ether was used as described merely to hasten immobilization of the animal before the melanophores could expand. The animal was held against the thin side of the box by means of pins thrust into the paraffin.

Usually transmitted light from the mirror or directly from a lamp was used, but reflected light with or without transmitted light was sometimes used. Sufficient light could be transmitted through the tail of a tadpole to permit the use of an oil-immersion lens, but the best results

were obtained by means of a 4 mm. lens and a 10 × to 18 × ocular. Magnifications up to nearly 1000 diameters were frequently obtained. The animals remained alive in the glass box for over 24 hours, so that very satisfactory records were obtained up to that length of time.

A vertical drawing board was used to permit the employment of a camera lucida. Individual pigment cells were located and followed for several hours. Hence by making drawings at frequent intervals, the exact courses of the cells could be followed. The movement was too slow to be observed except by means of a series of drawings.

In following the movements of melanophores, the neighboring epidermal cells were observed at the same time. It was found that the

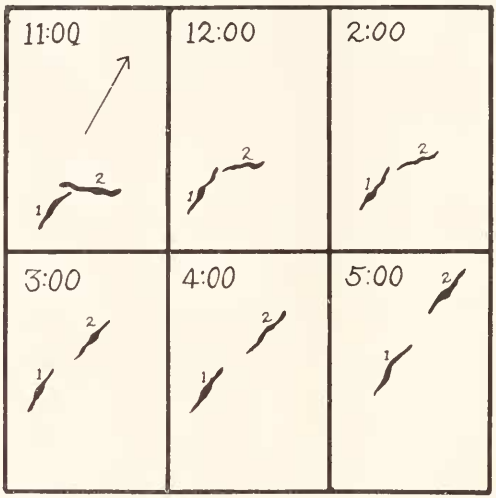

FIG. 7. First section of series of camera lucida drawings showing the paths taken by epidermal melanophores as they are carried toward a wound, coming to lie with long axes nearly perpendicular to the edge of the wound. The arrow indicates the direction of movement.

melanophores did not orient themselves irrespective of epidermal cells but moved with them and at approximately the same rate. Several investigators have concluded, from tissue culture studies, that melanophores possess the power of self-movement. The above described observations afford no ground for doubting that melanophores do have the power of self-movement, but there is every reason to believe that, under the conditions of the experiments in question, they are no more active than the other epidermal cells and that their radial arrangement depends upon the movement of the epidermal cells and not upon the activity of the melanophores themselves. Figures 7 and 8 are series of camera lucida drawings of groups of pigment cells which were near

healing wounds. The drawings show the successive positions of the melanophores as they became more nearly perpendicular to the edges of the wounds. The time intervals between successive drawings are indicated.

With regard to radial arrangement of pigment cells, there was a striking difference between auto- and homoiotransplants. There was never extensive radial arrangement around autotransplants. This is not strange, however, since in autotransplants there is little or no migration of epidermis after the epidermis of the host meets that of the graft. In fact, this is further evidence in support of the proposed explanation for radial arrangement.

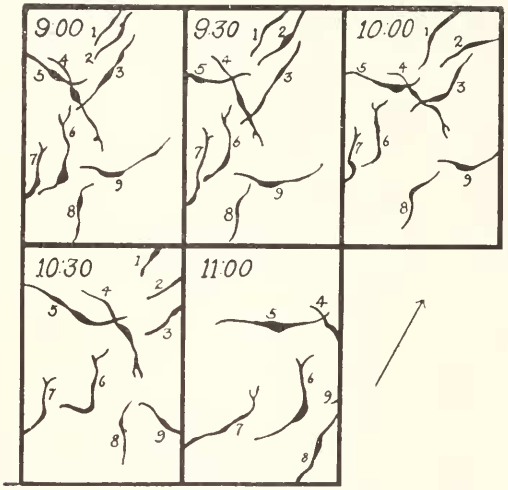

FIG. 8. Second section of a series of camera lucida drawings showing the paths taken by epidermal melanophores as they are carried toward a wound, coming to lie with long axes nearly perpendicular to the edge of the wound. The arrow indicates the direction of movement.

The writer wishes to express his sincere appreciation to Dr. H. W. Rand for his advice and aid throughout the period of this work.

SUMMARY

1. Immediately following the occurrence of wounds in the skin of tadpoles of *Rana clamitans,* the surrounding epidermis moves to cover the area. Wounds 5 mm. across may be covered in from 6 to 24 hours. The advancing epidermis is reduced to one or two cells in thickness, thus covering a larger area than previously. The epidermal cells become considerably elongated in the direction of movement. After the

advancing edges have met, the cells resume their previous shape. Subsequent mitosis restores the original thickness of the layer. There is considerable evidence for stereotropism in epidermal migration.

2. In wounds which cause a deficiency in the dermis, the epidermis thickens over the wound area until the smooth contour of the body-surface is restored.

3. In the region surrounding a healing wound there is temporarily a deficiency in the number of epidermal pigment cells due to their movement into the wound.

4. Autotransplants of white ventral skin transferred to the pigmented backs of tadpoles retained their specificity. They remained unpigmented for more than 100 days. Lateral and dorsal autotransplants in which the patches of skin were rotated through 90 or 180° also retained their specificity. There was no change in the pigmentation or the cellular structure of such grafts.

5. In all cases where homoiotransplants of white ventral skin were transferred to the pigmented backs of tadpoles the region of the graft became pigmented. Epidermal pigment appeared at the edge of the graft in an average of 9 days after the graft was made, and in the course of about 6 more days had extended to the center of the patch. Dermal pigment entered the graft area many days after the epidermal pigment had completely covered the patch.

6. The initial pigmentation of grafts results from a replacement of the graft epidermis by that of the host. The pigment cells are carried along by the host epidermis as it covers the patch. There is considerable evidence that the graft epidermis is destroyed by phagocytic action of the host epidermis. However, the epidermal layers of graft and host always unite immediately after the graft is made, indicating some initial affinity between the two layers.

7. Patches of skin which were grafted onto the ventral side of the body always degenerated. The ventral side of the body is perhaps an unfavorable place for grafts to receive nourishment.

8. The replacement of the epidermis of a graft by host epidermis is, essentially, a process of delayed wound healing.

9. In nearly all cases grafts, soon after transplantation, became reddened as the result of an enlargement of the dermal blood vessels of the graft. There is considerable evidence that the enlargement of the blood vessels is due to blood pressure which mechanically stretches the vessels in the absence of the normal tonus, the nerve connections having been cut at the time of transplantation. Later, and probably in consequence of restoration of nerve connections, the dermal vessels became reduced to normal proportions.

10. Radial arrangement of the epidermal melanophores occurs around homoiotransplants and skin wounds—that is, wherever there is translatory movement of the epidermis. In any migrating epidermis in which the melanophores are expanded, their long axes tend to become parallel to the direction of migration. The position of the individual melanophore, in radial or parallel alignment, is a consequence of the movement of the epidermal cells near a wound and is not due to independent orientation of the melanophores. The epidermal cells nearest the wound move first and most rapidly, therefore tending to rotate the elongated melanophores into the line of movement of the epidermis. Since the movement of the epidermis is centripetal, the melanophores become arranged radially around the point toward which the epidermis moves.

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